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Evaluation of the Cobas TaqMan MTB Test for the Detection of *Mycobacterium tuberculosis* Complex According to Acid-Fast-Bacillus Smear Grades in Respiratory Specimens

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We evaluated the performance of the Cobas TaqMan MTB test (Roche Diagnostics, Basel, Switzerland), stratified by acid-fast bacilli (AFB) smear grades. The sensitivity of this test in smear-positive specimens was >95% in all grades, while that in trace and negative specimens was 85.3% and 34.4%, respectively.

The rapid detection of *Mycobacterium tuberculosis* complex DNA from respiratory specimens and the ability to differentiate *M. tuberculosis* complex from nontuberculous mycobacteria (NTM) are important for the early diagnosis of pulmonary tuberculosis and the prompt use of adequate antibiotics (1–3). The direct detection of *M. tuberculosis* complex DNA by PCR-based assays has become an important part of the rapid diagnosis of tuberculosis (4). Numerous molecular assays for the rapid detection of *M. tuberculosis* complex DNA have been developed. The Cobas TaqMan MTB test (Cobas MTB test) (Roche Diagnostics, Basel, Switzerland) is one of the most widely used real-time PCR assays. It uses TaqMan hydrolysis probes and primers that bind to a highly conserved and specific region of the 16S rRNA sequence.

Recently, various rates of detection by different PCR methods, based by smear grade, were noted (5–7). The aim of this study was to investigate whether this observation extends to the Cobas MTB test and to investigate its diagnostic accuracy when stratified by acid-fast bacilli (AFB) smear grades.

This study was conducted at a tertiary care hospital in Seoul, South Korea, and was approved by the institutional review board of that hospital. A total of 6,852 Cobas MTB test results for respiratory specimens from April 2013 to June 2014 were retrospectively reviewed. Microbiological tests, including AFB smear and mycobacterial culture, were simultaneously performed for all specimens.

The respiratory specimens were processed with 2% *N*-acetyl–L-cysteine-sodium hydroxide (NALC-NaOH), followed by centrifugation at 3,000 × *g* for 20 min. The AFB smears were performed with an auramine-rhodamine fluorescent stain and subsequently confirmed by Ziehl-Neelsen staining. The staining results were graded according to the American Thoracic Society/Centers for Disease Control and Prevention (ATS/CDC) guidelines, as follows (8): no AFB seen, no bacilli in 300 fields; trace, 1 to 2 bacilli in 300 fields; 1+, 1 to 9 bacilli in 100 fields; 2+, 1 to 9 bacilli in 10 fields; 3+, 1 to 9 bacilli in 1 field; and 4+, >9 bacilli in 1 field. The specimens with the no AFB seen and trace grades were defined as smear negative, and those graded 1+ to 4+ were defined as smear positive. All patient specimens were cultured on both solid and liquid medium for 6 weeks. The positive cultures were confirmed by both the presence of cord formation and by MPT64 antigen testing (SD Bioline TB Ag MPT64 rapid assay; Standard Diagnostics, Inc., Yongin, South Korea). If any of these tests yielded a negative result, an *rpoB*-specific PCR test using the MTB-ID V3 kit (YD Diagnostics, Yongin, South Korea) was performed to differentiate between *M. tuberculosis* and NTM. The Cobas MTB test was performed according to the manufacturer's instructions (9). Conventional culture was considered the reference standard for performing calculations.

After the exclusion of 80 samples with contaminated culture results and/or invalid Cobas MTB test results, a total of 6,772 respiratory specimens from 5,604 patients were available for analysis. A total of 269 specimens were culture positive, while the remaining 6,503 specimens were culture negative (Table 1). Among *M. tuberculosis* culture-positive specimens, 110 specimens (40.9%) were smear positive, and the remaining 159 were smear negative. Of the culture-negative samples, 6,371 specimens (98.0%) were smear negative.

The Cobas MTB test yielded 212 positive results, of which 180 were positive by PCR and culture. A total of 6,471 specimens were negative by PCR and culture (Table 2). The overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Cobas MTB test were 66.9% (95% confidence interval [CI], 60.9 to 72.4%), 99.5% (95% CI, 99.3 to 99.7%), 84.9% (95% CI, 79.2 to 89.3), and 98.6% (95% CI, 98.3 to 98.9), respectively. The sensitivity was 98.2% for the smear- and culture-positive specimens and 45.3% for the smear-negative and culture-positive specimens.

In 242 smear-positive specimens, 110 *M. tuberculosis* and 123 NTM isolates were identified, while the remaining 9 specimens showed no growth. The sensitivities of the Cobas MTB test for

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TABLE 1 Comparison of the Cobas TaqMan MTB test and culture results stratified by AFB smear grade[a]

Smear grade	No. with culture result: Positive	Total negative (no growth/ NTM)	No. with Cobas TaqMan MTB test result: Positive	Negative	Total no.
Negative	159	6,371 (5,828/543)	99	6,431	6,530
No AFB seen	125	6,265 (5,791/484)	63	6,327	6,390
Trace	34	106 (47/59)	36	104	140
Positive	110	132 (9/123)	113	129	242
1+	61	82 (6/76)	62	81	143
2+	17	25 (2/23)	19	23	42
3+	13	15 (0/15)	13	15	28
4+	19	10 (1/9)	19	10	29
Total	269	6,503 (5,837/666)	212	6,560	6,772

[a] AFB, acid-fast bacilli.

these samples were 96.7% (95% CI, 87.6 to 99.4%) and 100% for grades 1+ and ≥2+, respectively. The NPVs were 97.5% (95% CI, 90.5 to 99.6%) and 100% for 1+ and ≥2+, respectively. In all AFB grades, the specificities were >90% (Table 2). The PPVs were 95.2% (95% CI, 85.6 to 98.7%), 89.5% (95% CI, 65.5 to 98.2%), and 100% for grades 1+, 2+, and ≥3+, respectively.

In 6,530 smear-negative specimens, 159 *M. tuberculosis* and 543 NTM isolates were identified, while the remaining 5,828 specimens showed no growth. The sensitivities for the trace and no AFB seen specimens were 85.3% (95% CI, 68.2 to 94.4%) and 34.4% (95% CI, 26.3 to 43.5%), respectively, while the PPVs for the trace and no AFB seen specimens were 80.6% (95% CI, 63.4 to 91.2%) and 68.3% (95% CI, 55.2 to 79.1%), respectively. The specificities and NPVs were >90% and >95% for all AFB-negative grades, respectively (Table 2).

When stratified by culture results, the specificities in the specimens with NTM growth were >99%: 100% (95% CI, 96.2 to 100%) in smear-positive specimens, 100% (95% CI, 92.4 to 100%) in smear trace specimens, and 99.4% (95% CI, 98.0 to 99.8%) in no AFB seen specimens. The Cobas MTB test was able to effectively discriminate between *M. tuberculosis* and NTM isolates in the smear-positive specimens without cross-reactivity in the smear-negative samples.

Many prior studies have analyzed the performance of the Cobas MTB test by stratification between smear-positive or smear-negative specimens (9–16). As expected, the sensitivity of the Cobas MTB test was higher with smear-positive specimens than that with smear-negative specimens, consistent with previous reports (10, 13, 14, 16). However, it was notable that a sensitivity of about 85% was observed in the AFB trace specimens, which was significantly higher than that for the no AFB seen specimens.

Recently, Mareković et al. (7) demonstrated that real-time PCR was insufficiently reliable to exclude tuberculosis in smear-positive samples with few bacilli due to low sensitivity (52%) in the AFB 1+ samples, despite grading the smear results according to more stringent World Health Organization (WHO) criteria. The WHO classifications scanty and 1+ match the ATS/CDC 1+ and 2+ grades, respectively (17). However, the Cobas MTB test showed excellent performance in the smear-positive specimens, regardless of smear grade.

In this study, 32 specimens were *M. tuberculosis* PCR positive but *M. tuberculosis* culture negative, of which five were smear positive. An evaluation of patient clinical features, including chest symptoms, radiologic findings compatible with tuberculosis, culture results for additional specimens, and a history of antibiotics showed that 26 culture-negative specimens could be categorized as true positives for detecting *M. tuberculosis* DNA (Table 3). When considering the 26 discrepant results as clinically concordant results, the specificity and PPV of the Cobas MTB test were 100% for all smear-positive grades and trace specimens. Therefore, a single positive Cobas MTB test result in a respiratory specimen supports the diagnosis of tuberculosis in smear-positive samples.

In conclusion, the Cobas MTB test in conjunction with AFB smear and culture on respiratory specimens was helpful for the early diagnosis for tuberculosis. The Cobas MTB test exhibits excellent performance not only with high-grade smear-positive specimens but also with paucibacillary respiratory samples. Furthermore, the Cobas assay was able to effectively discrimi-

TABLE 2 Performance of the Cobas TaqMan MTB test stratified by AFB smear grade[a]

Smear grade	No. with PCR/culture result of: +/+	−/−	Performance (% [95% CI])[b] Sensitivity	Specificity	PPV	NPV
Negative	72/159	6,344/6,371	45.3 (37.4–53.4)	99.6 (99.4–99.7)	72.7 (62.7–81.0)	98.6 (98.3–98.9)
No AFB seen	43/125	6,245/6,265	34.4 (26.3–43.5)	99.7 (99.5–99.8)	68.3 (55.2–79.1)	98.7 (98.4–99.0)
Trace	29/34	99/106	85.3 (68.2–94.4)	93.4 (86.4–97.1)	80.6 (63.4–91.2)	95.2 (88.6–98.2)
Positive	108/110	127/132	98.2 (92.9–99.7)	96.2 (90.9–98.6)	95.6 (89.5–98.4)	98.4 (93.9–99.7)
1+	59/61	79/82	96.7 (87.6–99.4)	96.3 (88.9–99.1)	95.2 (85.6–98.7)	97.5 (90.5–99.6)
2+	17/17	23/25	100 (77.1–100)	92.0 (72.5–98.6)	89.5 (65.5–98.2)	100 (82.2–100)
3+	13/13	15/15	100 (71.7–100)	100 (74.7–100)	100 (71.7–100)	100 (74.7–100)
4+	19/19	10/10	100 (79.1–100)	100 (65.5–100)	100 (79.1–100)	100 (65.5–100)
Total	180/269	6,471/6,503	66.9 (60.9–72.4)	99.5 (99.3–99.7)	84.9 (79.2–89.3)	98.6 (98.3–98.9)

[a] AFB, acid-fast bacilli.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

TABLE 3 Analysis of 32 specimens positive by the Cobas TaqMan MTB test and negative for culture

No. of specimens	Detection of MTB complex by culture		Discrepancy analysis	Final interpretation
	Result	Smear grade		
1	Negative	2+	Tuberculosis patient receiving tuberculosis treatment	True positive
1	Negative	1+	Tuberculosis patient receiving tuberculosis treatment	True positive
4	Negative	Trace	Tuberculosis patients receiving tuberculosis treatment	True positive
6	Negative	No AFB seen[a]	Tuberculosis patients receiving tuberculosis treatment	True positive
1	NTM[b]	No AFB seen	Tuberculosis patient receiving tuberculosis treatment	True positive
2	Negative	1+	*M. tuberculosis* culture grown from another specimen	True positive
3	Negative	Trace	*M. tuberculosis* culture grown from another specimen	True positive
3	Negative	No AFB seen	*M. tuberculosis* culture grown from another specimen	True positive
1	NTM	No AFB seen	*M. tuberculosis* culture grown from another specimen	True positive
1	Negative	2+	Diagnosed with tuberculosis based on symptoms, radiologic findings, and response to antituberculosis medications	True positive
3	Negative	No AFB seen	Diagnosed with tuberculosis based on symptoms, radiologic findings, and response to antituberculosis medications	True positive
5	Negative	No AFB seen	NA[c]	False positive
1	NTM	No AFB seen	NA	False positive

[a] AFB, acid-fast bacilli.
[b] NTM, nontuberculous mycobacteria.
[c] NA, not applicable.

nate between *M. tuberculosis* and NTM, regardless of smear grade.

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REFERENCES

1. **Dye C, Williams BG.** 2010. The population dynamics and control of tuberculosis. Science **328:**856–861. http://dx.doi.org/10.1126/science.1185449.
2. **Snider DE, Jr, La Montagne JR.** 1994. The neglected global tuberculosis problem: a report of the 1992 World Congress on Tuberculosis. J Infect Dis **169:**1189–1196. http://dx.doi.org/10.1093/infdis/169.6.1189.
3. **Lai CC, Tan CK, Chou CH, Hsu HL, Liao CH, Huang YT, Kao CL, Luh KT, Hsueh PR.** 2010. Increasing incidence of nontuberculous mycobacteria, Taiwan, 2000–2008. Emerg Infect Dis **16:**294–296. http://dx.doi.org/10.3201/eid1602.090675.
4. **Laraque F, Griggs A, Slopen M, Munsiff SS.** 2009. Performance of Nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clin Infect Dis **49:**46–54. http://dx.doi.org/10.1086/599037.
5. **Wu TL, Chia JH, Kuo AJ, Su LH, Wu TS, Lai HC.** 2008. Rapid identification of mycobacteria from smear-positive sputum samples by nested PCR-restriction fragment length polymorphism analysis. J Clin Microbiol **46:**3591–3594. http://dx.doi.org/10.1128/JCM.00856-08.
6. **Chia JH, Wu TL, Su LH, Kuo AJ, Lai HC.** 2012. Direct identification of mycobacteria from smear-positive sputum samples using an improved multiplex polymerase chain reaction assay. Diagn Microbiol Infect Dis **72:**340–349. http://dx.doi.org/10.1016/j.diagmicrobio.2011.12.008.
7. **Marekovi I, Bošnjak Z, Plečko V.** 2014. Direct identification of mycobacteria from smear-positive samples using real-time polymerase chain reaction. Int J Tuberc Lung Dis **18:**978–980. http://dx.doi.org/10.5588/ijtld.13.0042.
8. **The American Thoracic Society.** 2000. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med **161:**1376–1395. http://dx.doi.org/10.1164/ajrccm.161.4.16141.
9. **Kim JH, Kim YJ, Ki CS, Kim JY, Lee NY.** 2011. Evaluation of Cobas TaqMan MTB PCR for detection of *Mycobacterium tuberculosis*. J Clin Microbiol **49:**173–176. http://dx.doi.org/10.1128/JCM.00694-10.
10. **Yang YC, Lu PL, Huang SC, Jenh YS, Jou R, Chang TC.** 2011. Evaluation of the Cobas TaqMan MTB test for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J Clin Microbiol **49:**797–801. http://dx.doi.org/10.1128/JCM.01839-10.
11. **Cho SY, Kim MJ, Suh JT, Lee HJ.** 2011. Comparison of diagnostic performance of three real-time PCR kits for detecting *Mycobacterium* species. Yonsei Med J **52:**301–306. http://dx.doi.org/10.3349/ymj.2011.52.2.301.
12. **Tortoli E, Urbano P, Marcelli F, Simonetti TM, Cirillo DM.** 2012. Is real-time PCR better than conventional PCR for *Mycobacterium tuberculosis* complex detection in clinical samples? J Clin Microbiol **50:**2810–2813. http://dx.doi.org/10.1128/JCM.01412-12.
13. **Park KS, Kim JY, Lee JW, Hwang YY, Jeon K, Koh WJ, Ki CS, Lee NY.** 2013. Comparison of the Xpert MTB/RIF and Cobas TaqMan MTB assays for detection of *Mycobacterium tuberculosis* in respiratory specimens. J Clin Microbiol **51:**3225–3227. http://dx.doi.org/10.1128/JCM.01335-13.
14. **Lee MR, Chung KP, Wang HC, Lin CB, Yu CJ, Lee JJ, Hsueh PR.** 2013. Evaluation of the Cobas TaqMan MTB real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory specimens. J Med Microbiol **62:**1160–1164. http://dx.doi.org/10.1099/jmm.0.052043-0.
15. **Antonenka U, Hofmann-Thiel S, Turaev L, Esenalieva A, Abdulloeva M, Sahalchyk E, Alnour T, Hoffmann H.** 2013. Comparison of Xpert MTB/RIF with ProbeTec ET DTB and Cobas TaqMan MTB for direct detection of *M. tuberculosis* complex in respiratory specimens. BMC Infect Dis **13:**280. http://dx.doi.org/10.1186/1471-2334-13-280.
16. **Lim J, Kim J, Kim JW, Ihm C, Sohn YH, Cho HJ, Kim J, Koo SH.** 2014. Multicenter evaluation of Seegene Anyplex TB PCR for the detection of *Mycobacterium tuberculosis* in respiratory specimens. J Microbiol Biotechnol **24:**1004–1007.
17. **World Health Organization.** 2013. Definitions and reporting framework for tuberculosis—2013 revision. World Health Organization, Geneva, Switzerland. http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf.